

WHAT IS CLAIMED:

1. A method for discriminating dysplastic cells non-dysplastic cells in a biological sample comprising determining in the biological sample the co-expression of at least two marker molecules in at least one single cell, wherein at least one marker molecule is an INK4a gene product, which is an expression product encoded by the INK4a gene, and at least one further marker molecule is a cell proliferation marker, wherein the over-expression of the at least one INK4a gene product and expression of the at least one cell proliferation marker for active cell proliferation at a detectable level within said single cell is indicative of the dysplastic state of the cell, and the over-expression of the at least one INK4a gene product and expression of the at least one cell proliferation marker for senescence, terminal cell differentiation, apoptosis or cell cycle arrest at a detectable level within said single cell is indicative of the non-dysplastic state of the cell.
2. The method according to claim 1, wherein a set of two or more cell proliferation markers is detected.
3. The method according to claim 1, wherein the INK4a gene product has a molecular weight between 13 and 19 kDa.
4. The method according to claim 1, wherein the INK4a gene product is selected from the group consisting of p16^{INK4a} and p14ARF.
5. The method according to claim 1, wherein the cell proliferation marker is selected from the group consisting of a proliferation marker necessary for the maintenance of cell proliferation, a proliferation marker engaged in DNA replication, a proliferation marker being or encoding a member of the processive replication fork, a senescence marker, a cell cycle arrest marker and an apoptosis marker.
6. The method according to claim 5, wherein the proliferation marker necessary for the maintenance of cell proliferation is a molecule selected from the group consisting of Ki67 molecules, Ki-S5 molecules and Ki-S2 molecules.

7. The method according to claim 6, wherein the proliferation marker engaged in DNA replication is selected from the group consisting of helicases or subunits thereof, cell division cycle molecules, phosphatase molecules and kinase molecules.
8. The method according to claim 7, wherein the helicases or subunits thereof are selected from the group consisting of MCM2, MCM3, MCM4, MCM5, MCM6, MCM7 and HELAD1.
9. The method according to claim 7, wherein the cell division cycle molecules, kinases molecules and phosphatases molecules are selected from the group consisting of CDC6, CDC7 protein kinase, Dbf4, CDC14 protein phosphatase, CDC45 and MCM10.
10. The method according to claim 5, wherein the member of the processive replication fork is selected from the group consisting of PCNA and POLD.
11. The method according to claim 1, wherein the INK4a gene product is a polypeptide or a nucleic acid molecule.
12. The method according to claim 1, wherein additionally at least one further marker molecule is detected for improvement of the assessment of diagnosis or prognosis.
13. The method according to claim 12, wherein the further marker molecule is at least one further proliferation marker molecule.
14. The method according to claim 12, wherein the further marker molecule is selected from the group consisting of a senescence marker, an apoptosis marker, a cell cycle arrest marker, a marker for terminal differentiation of cells, a marker for viral infection, a marker for viral activity, a cell cycle regulatory protein, a gene-product necessary for the maintenance of cell proliferation, a gene-product engaged in DNA replication, and a gene product being a member of the processive replication fork.
15. The method according to claim 12, wherein additionally a cytological staining procedure employing at least one dye selected from the group consisting of DAPI, Quinacrin, Chromomycin, Azan, Acridin-orange, Hematoxylin, Eosin, Sudan-red,

Toluidin-blue, and Thionin, or a staining method selected from the group consisting of Pap-staining, Giemsa-staining, Hematoxylin-Eosin staining, van-Gieson-staining, Schiff-staining, staining via metal precipitates, Turnbulls-blue-staining and staining via metal cyanides, is applied.

- 5 16. The method according to claim 1, wherein the dysplastic cells are cells of a cancerous or precancerous lesion.
17. The method according to claim 16, wherein the dysplastic cells are cells of a dysplasia being associated with a papilloma virus.
- 10 18. The method according to claim 17, wherein the papilloma virus is a high risk human papilloma virus selected from the group consisting of HPV16, HPV18, HPV31, HPV 33, HPV35, HPV 39, HPV 45, HPV 51, HPV 52, HPV56, HPV 58, HPV 59, HPV 66 and HPV 68.
- 15 19. The method according to claim 16, wherein the lesion is selected from the group consisting of a lesion of the anogenital tract, a lesion of the respiratory tract and a lesion of the skin and it's appendages.
- 20 20. The method according to claim 19, wherein the lesion is selected from the group consisting of a lesion of the uterine cervix, a lesion of the vagina, a lesion of the vulva, a lesion of the penis, a lesion of the anus, a lesion of the rectum, a lesion of the bronchic tree, a lesion of the lung, a lesion of the peritoneal space, a lesion of the naso-pharyngeal space, a lesion of the oral cavity or and a lesion of the skin.
21. The method according to claim 1, wherein the biological sample is a sample containing cells originating from the anogenital tract, from the respiratory tract or from the skin and its appendages.
- 25 22. The method according to claim 21, wherein the biological sample is obtained from the uterine cervix, the vagina, the vulva, the penis, the anus, the rectum, the bronchic tree, the lung, the naso-pharyngeal space, the oral cavity or the skin.

23. The method according to claim 22, wherein the biological sample is a cytological or histological preparation.
24. The method according to claim 1, wherein the determination of the INK4a gene product and/or the cell proliferation marker molecules is performed using at least one probe specifically recognising at least one of the respective molecules to be detected.
25. The method according to claim 24, wherein the at least one probe is detectably labelled with at least one label.
26. The method according to claim 25, wherein the label is selected from the group consisting of a radioisotope, a bioluminescent compound, a chemiluminescent compound, a fluorescent compound, a metal chelate, and an enzyme.
27. The method according to claim 24, wherein the probe is a protein and/or a nucleic acid.
28. The method according to claim 27, wherein the protein is an antibody directed against a INK4a encoded gene product or a cell proliferation marker gene product.
29. The method according to claim 28, which comprises an immuno-cytochemical staining procedure.
30. The method according to claim 25, wherein the probe is a nucleic acid specifically hybridizing to an INK4a gene product or a cell proliferation marker gene product.
31. The method according to claim 30, which comprises an in situ hybridization reaction.
32. The method according to claim 30, which comprises a nucleic acid amplification reaction.
33. The method according to claim 32, wherein the nucleic acid amplification reaction is PCR, NASBA or LCR.

34. The method according to claim 27, wherein the determination using nucleic acid probes and polypeptide probes are carried out simultaneously.
35. A kit comprising at least one or more probes for the detection of the presence or absence and/or the level of the over-expression of at least one INK4a gene product
5 and at least one cell proliferation marker gene product in biological samples.
36. The kit according to claim 35, wherein the INK4a gene products are selected from the group consisting of p16^{INK4a} and p14ARF.
37. (Currently Amended) The kit according to claim 35, wherein the cell proliferation marker gene products are selected the group consisting of CDC6, MCM3, MCM3,
10 MCM4, MCM5, MCM6, MCM7, CDC7 protein kinase, Dbf4, CDC14 protein phosphatase, CDC45 and MCM10, Ki67, Ki-S2, PCNA and POLD.
38. The kit according to claim 35 furthermore comprising at least one of the following:
- a. a p16^{INK4a} sample for carrying out a positive control reaction,
 - b. a p14ARF sample for carrying out a positive control reaction,
 - 15 c. a Ki67 sample for carrying out a positive control reaction,
 - d. a Ki-S2 sample for carrying out a positive control reaction,
 - e. an MCM5 sample for carrying out a positive control reaction,
 - f. an MCM2 sample for carrying out a positive control reaction,
 - g. a PCNA sample for carrying out a positive control reaction,
 - 20 h. reagents for detection of the presence or absence and/or the level of p16^{INK4a},
 - i. reagents for detection of the presence or absence and/or the level of p14ARF,
 - j. reagents for detection of the presence or absence and/or the level of Ki67,
 - k. reagents for detection of the presence or absence and/or the level of Ki-S2,
 - 25 l. reagents for detection of the presence or absence and/or the level of MCM5,

- m. reagents for detection of the presence or absence and/or the level of MCM2,
- n. reagents for detection of the presence or absence and/or the level of PCNA,
- o. one or more samples of INK4a gene-products for carrying out positive control reactions,
- 5 p. one or more samples of cell proliferation marker gene-products for carrying out positive control reactions,
- q. one or more reagents for the detection of the presence or absence and/or the level of other INK4a gene products, or
- 10 r. and one or more reagents for the detection of the presence or absence and/or the level of other cell proliferation marker gene products.